NEW CAPILLARY ELECTROPHORESIS METHODS FOR THE ANALYSIS OF PARALYTIC SHELLFISH POISONING TOXINS

By

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Declaration

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Statement of co-authorship

The following people and institutions contributed to the publication of work undertaken as part of this thesis:

Author details and their roles:

Paper 1, **<** Keyon, A.S.A., Guijt, R.M., Gaspar, A., Kazarian, A.A., Nesterenko, P.N., Bolch, C.J. and Breadmore, M.C. **Capillary Electrophoresis for the Analysis of Paralytic Shellfish Poisoning Toxins in Shellfish: Comparison of Detection Methods**, *Electrophoresis*, 35, (2014) 1496-1503**>*:***

This paper comprises the majority of Chapter 2

Aemi Abdul Keyon was the primary author (70%) and conducted all the experiments, analysed data and wrote the manuscript. The co-authors contributed a total of 30% to the published work. Michael Breadmore, Rosanne Guijt and Chris Bolch contributed to idea, its formalisation and development. Andras Gaspar, Artaches Kazarian and Pavel Nesterenko offered experimental assistance. All co-authors assisted with refinement and presentation.

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Aemi Abdul Keyon was the primary author (75%) and conducted all the experiments, analysed data and wrote the manuscript. The co-authors contributed a total of 25% to the published work. Michael Breadmore, Rosanne Guijt and Chris Bolch contributed to the concept, development, refinement and presentation.

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Aemi Abdul Keyon was the primary author (75%) and conducted all the experiments, interpreted the results and wrote the manuscript. The co-authors contributed a total of 25% to work. Michael Breadmore and Rosanne Guijt contributed to the idea, its formalisation, development and presentation. Chris Bolch assisted with refinement and presentation.

We the undersigned agree with the above stated "proportion of work undertaken" for each of the above published (or submitted) peer-reviewed manuscripts contributing to this thesis:

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List of publications and presentations

Parts of research works described in this thesis have been or will be reported in the following publications and presentations:

1. Keyon, A.S.A., Guijt, R.M., Gaspar, A., Kazarian, A.A., Nesterenko, P.N., Bolch, C.J. and Breadmore, M.C. **Capillary Electrophoresis for the Analysis of Paralytic Shellfish Poisoning Toxins in Shellfish: Comparison of Detection Methods**, *Electrophoresis*, 35, (2014) 1496-1503. DOI: 10.1002/elps.201300353. **(Chapter 2)**

2. Keyon, A.S.A., Guijt, R.M., Bolch, C.J. and Breadmore, M.C., **Transient Isotachophoresis-Capillary Zone Electrophoresis with Contactless Conductivity and Ultraviolet Detection for the Analysis of Paralytic Shellfish Toxins in Mussel Sample**. *J. Chromatogr. A*, (2014) 1364, 295-302. DOI: 10.1016/j.chroma.2014.08.074. **(Chapter 3)**

3. Keyon, A.S.A., Guijt, R.M., Bolch, C.J. and Breadmore, M.C., **Droplet Microfluidics for Post-Column Reactions in Capillary Electrophoresis**, Analytical Chemistry (2014), Article in press, DOI: 10.1021/ac5033963. **(Chapter 4)**

4. Keyon, A.S.A., Guijt, R.M., Bolch, C.J. and Breadmore, M.C., **Liquid Chromatography and Capillary Electrophoresis for The Analysis of Major Phycotoxins: A Review** (manuscript in preparation)

5. Keyon, A.S.A., Guijt, R.M., Bolch, C.J. and Breadmore, M.C., **Capillary Electrophoresis for the Analysis of Paralytic Shellfish Poisoning Toxins in Shellfish: A Preliminary Study,** UTAS Postgraduate Conference, University of Tasmania, Australia, 6-7 September 2012.
6. Keyon, A.S.A., Guijt, R.M., Bolch, C.J. and Breadmore, M.C., **Capillary Electrophoresis for the Analysis of Paralytic Shellfish Poisoning Toxins in Shellfish: Comparison of Detection Methods**, The 28th International Symposium On Microscale Bioseparations, Shanghai, China, 21-24 October 2012.
7. Keyon, A.S.A., Guijt, R.M., Bolch, C.J. and Breadmore, M.C., **Capillary Electrophoresis for the Analysis of Paralytic Shellfish Poisoning Toxins in Shellfish: Comparison of Detection Methods**, 9th International Conference on Molluscan Shellfish Safety, Sydney, Australia, 17-22 March 2013.
8. Keyon, A.S.A., Guijt, R.M., Bolch, C.J. and Breadmore, M.C., **Transient Isotachophoresis-Capillary Zone Electrophoresis for The Analysis of Paralytic Shellfish Toxins in Mussel Sample**, 40th International Symposium on High-Performance Liquid-Phase Separations and Related Techniques (HPLC 2013), Hobart, Australia, 18-21 November 2013.
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List of Abbreviations

ACN	Acetonitrile
AOAC	Association of Official Analytical Chemists
AQC	6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate
ASP	Amnesic shellfish poisoning
AZA	Azaspiracid
BGE	Background electrolyte
CFP	Ciguatera fish poisoning
CZE	Capillary zone electrophoresis
C^4D	Capacitively coupled contactless conductivity detection
dcGTX2	decarbamoylgonyautoxin2
dcGTX3	decarbamoylgonyautoxin3
dcNEO	decarbamoylneosaxitoxin
dcSTX	decarbamoylsaxitoxin
DA	Domoic acid
DSP	Diarrhetic shellfish poisoning
DTX	Dinophysistoxin
EIA	Extracted ion electropherogram
ECD	Electrochemical detection
ELISA	Enzyme-linked immunosorbent assay
EOF	Electroosmotic flow
λ_{ex}	Excitation wavelength
λ_{em}	Emission wavelength

μ_{ep}	Electrophoretic mobility
μ_{eff}	Effective mobility
FLD	Fluorescence detection
GTX1	gonyautoxin1
GTX2	gonyautoxin2
GTX3	gonyautoxin3
GTX4	gonyautoxin4
GTX5	gonyautoxin5
GTX6	gonyautoxin6
He-Cd	Helium-Cadmium
HILIC	Hydrophilic interaction liquid chromatography
HPLC	High performance liquid chromatography
H_2O_2	Hydrogen peroxide
ITP	Isotachophoresis
LE	Leading electrolyte
LED	Light emitting diode
LIF	Laser induced fluorescence
LLE	Liquid-liquid extraction
LOD	Limit of detection
MEKC	Micellar electrokinetic chromatography
MRM	Multiple reactions monitoring
MS	Mass spectrometry
NSP	Neurotic shellfish poisoning
OA	Okadaic acid
OPA	o-phthaldialdehyde

Pt	Platinum
PTX	Pectenotoxin
PSP	Paralytic shellfish poisoning
PSTs	Paralytic shellfish toxins
RBA	Receptor binding assay
RP	Reversed phase
RSD	Relative standard deviation
SDS	Sodium dodecyl sulfate
SEF	Sensitivity enhancement factor
S/N	Signal-to-noise ratio
SPE	Solid phase extraction
TEFs	Toxicity equivalent factors
TE	Terminating electrolyte
tITP	Transient isotachophoresis
t_m	Migration time
UV	Ultraviolet
YTX	Yessotoxin

Abstract

Paralytic shellfish poisoning (PSP) toxins, or usually termed as paralytic shellfish toxins (PSTs), produced by marine and freshwater microalgae during algal blooms can accumulate in filter-feeding bivalve shellfish. Early detection of PSTs in shellfish is therefore important for food and public health safety. High performance liquid chromatography (HPLC) methods with pre- or post-column oxidation for fluorescence detection (FLD) and HPLC-mass spectrometry (MS) are the most widely used instrumental analytical methods for PSTs, but are not easily miniaturised for field-deployable portable analyser. Capillary electrophoresis (CE) can be developed as an alternative method as it is compatible with miniaturisation, making it an attractive method for a portable analyser for early on-site detection.

In order to develop appropriate portable instrumentation for CE of PSTs, it is necessary to develop appropriate methods. This was first done by developing CE methods with different detection techniques namely ultraviolet (UV), capacitively coupled contactless conductivity detector (C^4D), MS, and FLD - making this the first report of the use of C^4D and an improved FLD detection for various PSTs with CE. Due to the fact that most oxidised PSTs were neutral, micellar electrokinetic chromatography (MEKC) was used in combination with FLD. The capillary zone electrophoresis-UV (CZE-UV) and CZE-C^4D methods provided better resolution, selectivity and separation efficiency compared to CZE-MS and MEKC-FLD methods. However, CZE-UV and CZE-MS methods did not provide sufficient sensitivity to detect PSTs at the regulatory concentration limit, while CZE-C^4D and MEKC-FLD did show sensitivity below or close to the regulatory limit. The latter most portable methods were evaluated for the screening of PSTs in a naturally

contaminated mussel sample. MEKC-FLD was successfully used for PSTs screening in the periodate-oxidised sample, whilst CZE-C^4D method suffered from significant interferences from sample matrix; a result that motivated further investigation of an on-line preconcentration method to deal with the high conductivity sample matrix and improve the sensitivity.

Therefore, CZE with C^4D was examined with counter-flow transient isotachophoresis (tITP). The tITP system exploited the naturally high sodium concentration in mussel sample to act as a leading ion, in combination with one electrolyte acting as terminating electrolyte (TE) and background electrolyte (BGE). Optimisation of the BGE concentration, duration of counter-flow and injected sample volume suitable for tITP resulted in sensitivity enhancement of at least two-fold over CZE-C^4D method developed in the first body of work. In particular, the modest gain in sensitivity was achieved in the existence of a high concentration of sodium, a sample matrix property that was problematic in previous method. This allowed the analysis of PSTs in mussel sample at below or close to the regulatory concentration limit.

The pre-column periodate oxidation MEKC-FLD method described in the first body of work enabled direct screening of PSTs in shellfish sample; however some toxins produced multiple and/or identical oxidation products, affecting selectivity and specificity of the method. The findings initiated investigation of CE with droplet microfluidic post-column reaction system for the separation and FLD of PSTs. The concept was that PSTs were separated using CZE and electrophoretically transferred into droplets segmented by oil. Formation of droplets and electrical connection in the CE-droplet microfluidic system were first evaluated. Depending on the total flow rate of both aqueous and oil phases, nL-sized droplets could be formed having

frequencies between 0.7-3.7 Hz. The use of an off-the-shelf micro cross for positioning a salt bridge across the droplet flow from the separation capillary outlet enabled the compartmentalisation of the analytes while maintaining the electrical connection. Further, the potential of the system was investigated for post-column oxidation of PSTs. Compartmentalised in the droplets, PSTs reacted with periodate oxidant already present in the droplets, in which only a single peak for each PST was detected by FLD.

Given that the general objective of this research study is to develop suitable CE methods that can be implemented for on-site PSTs detection, the potentials of the developed methods compatible with miniaturisation and portability have been demonstrated. The CE methods with different detection techniques, combined with an on-line preconcentration and ability to be coupled with post-column reaction indicates the versatility of CE as alternative analytical method for PSTs.

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